UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: February 27, 2013

SUBJECT: Mancozeb, Immunotoxicity study in Rats

PC Code: 014504
Decision No.: NA
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0056583
MRID No.: 48794801

DP Barcode: 402785
Registration No.: N/A
Regulatory Action: N/A
Submission No.: N/A
CAS No.: 8018-01-7
40 CFR: N/A

FROM: **Yung G. Yang, Pb.D.**
Risk Assessment Branch VI
Health Effects Division (7509 P)

THROUGH: **Felecia Fort, Chief**
Risk Assessment Branch VI
Health Effects Division (7509 P)

TO: **Christina Scheltema**
RMIB3
Pesticide Re-Evaluation Division (7508P)
And
Michael Metzger, Chief
Risk Assessment Branch VII
Health Effects Division (7509P)

I. CONCLUSIONS

The immunotoxicity study in rats for Mancozeb (MRID 48794801) has been reviewed. It is classified as acceptable/guideline and satisfies guideline requirements for an immunotoxicity study (OPPTS 870.7800).

II. BACKGROUND and ACTION REQUESTED

An immunotoxicity study on Mancozeb (MRID 48794801) has been submitted. RAB VI was asked to review and prepare a DER for this study.

III. RESULTS AND DISCUSSION

The immunotoxicity study in rats for Mancozeb (MRID 48794801) has been reviewed. The DER is attached and an executive summary is as follows:

EXECUTIVE SUMMARY: In an immunotoxicity study (MRID 48794801), Mancozeb (84.8 % w/w., Lot no. ZA0344n615, TSN 300199) was administered to male Crl:CD(SD) rats (10/dose) in the diet at dose levels of 0, 50, 200, or 1000 ppm (equivalent to 0, 4.1, 16.1, or 81.0 mg/kg/day, respectively) for 29 days. The positive control group (10 males) was administered 20 mg/kg bw/day of cyclophosphamide (CP) by intraperitoneal injection on study Days 24-28. During the study, clinical condition, bodyweight, food and water consumption, organ weight, and macroscopic pathology were evaluated. On the study Day 24, all animals in all groups received a single intravenous dose of sheep red blood cells (4 x 10^8 SRBC/mL, 0.5 mL/animal) in isotonic saline. At sacrifice, selected organs were removed and weighed (liver, spleen and thymus). The anti-SRBC IgM concentration was measured with Enzyme Linked Immunosorbent Assay (ELISA).

There were no premature deaths and no treatment-related clinical signs. No treatment related effects on food and water consumption, hematology parameters, and gross pathology in all treated groups. When compared to the animals in control group, animals in 1000 ppm group had slight treatment-related decrease in body weight (↓2.6 – 4.1%) from study Days 13-29, and body weight gain (↓ 8.5 – 10.8%) from study Days 7-29. The absolute and relative liver weights were increased (↑12.5% and 17.3%, respectively) and the absolute and relative thyroid weights were increased (↑20.0% and 25.6%, respectively). There were no treatment related effects on spleen and thymus weights in treated groups. Positive control group treated with cyclophosphamide had decreased body weight and body weight gain (↓3.8% and 8.6%, respectively) on study Day 29. In this group absolute and relative spleen weights were significantly decreased (↓50.1% and 48.2%, respectively); absolute and relative thymus weights were also significantly decreased (↓78.7% and 78.0%, respectively).

The systemic LOAEL was 1000 ppm (equivalent to 81mg/kg/day) based on significant increases of absolute and relative liver and thyroid weights. The systemic NOAEL was 200 ppm (equivalent to 16.1 mg/kg/day).

There were no statistically significant differences in anti-SRBC IgM levels in treated groups when compare to the vehicle control group. High inter-individual variability was noted in all the treatment groups as well as in the control group. Evaluation of the individual animal data of this study did not show any trend or distribution that would demonstrate significant suppression of anti-SRBC antibody response. Positive control group had statistically significant decrease in the anti SRBC IgM levels. This confirmed

the ability of the test system to detect immuno-suppressive effects and confirmed the validity of the study design.

The Natural Killer (NK) cells activity was not evaluated in this study. The toxicology database for Mancozeb does not reveal any evidence of immunotoxicity. The overall weight of evidence suggests that the chemical does not directly target the immune system. Under HED guidance a NK cell activity assay is not required at this time.

The NOAEL for immunotoxicity was 1000 ppm (equivalent to 81 mg/kg/day), the highest dose tested. The immunotoxicity LOAEL was not established.

This immunotoxicity study is classified **acceptable/guideline** and satisfy the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in rats.

EPA Primary Reviewer: Khin Swe Oo, MD, DABT **Signature:**
TEB, Health Effects Division (7509P) **Date:** 2/27/2013
EPA Secondary Reviewer: Yung G. Yang, Ph.D. **Signature:**
Risk Assessment Branch VI, Health Effects Division (7509P) **Date:** 2/27/2013.

Template version 09/11

TXR #: 0056583

DATA EVALUATION RECORD

STUDY TYPE: Immunotoxicity [dietary] - Rat OPPTS 870.7800

PC CODE: 014504 **DP BARCODE:** D402785

TEST MATERIAL (PURITY): Mancozeb (84.8 %, w.w.)

SYNONYMS: ((1,2-Ethanediylbis(carbamodithioato))(2-)manganese mixture with ((1,2-ethanediylbis(carbamodithioate))(2-) zinc; Manganese ethylenebis(dithiocarbamate)(polymeric) complex with zinc salt.

CITATIONs: Boverhof D.R., Marshall V.A., Golden R.M. (2012). Mancozeb: Assessment of Immunotoxic Potential Using the Sheep Red Blood Cell Assay After 28-Day Dietary Exposure to Male Crl:CD(SD) Rats. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674. Project ID.111143. March 30, 2012. MRID# 48794801. Unpublished.

Ahharwal M. (2012). Assessment of the Immunotoxic Potential of Mancozeb. Dow AgroSciences Ltd, European Development Center, 3 Milton Park, Ahongdon, Oxfordshire, UK OX14 4RN. Study No. MZ-WOE-2012. April 5, 2012. MRID# 48806801. Unpublished.

SPONSORs: Mancozeh Task Force, c/o Edward M.Ruckert, 600 13th Street N.W., Washington, DC 20005.

EXECUTIVE SUMMARY: In an immunotoxicity study (MRID #48794801), Mancozeb (84.8 % w/w., Lot no. ZA0344n615, TSN 300199) was administered to male Crl:CD(SD) rats (10/dose) in the diet at dose levels of 0, 50, 200, or 1000 ppm (equivalent to 0, 4.1, 16.1, or 81.0 mg/kg/day, respectively) for 29 days. The positive control group (10 males) was administered 20 mg/kg bw/day of cyclophosphamide (CP) by intraperitoneal injection on study Days 24-28. During the study, clinical condition, bodyweight, food and water consumption, organ weight, and macroscopic pathology were evaluated. On the study Day 24, all animals in all groups received a single intravenous dose of sheep red blood cells (4×10^8 SRBC/mL, 0.5 mL/animal) in isotonic saline. At sacrifice, selected organs were removed and weighed (liver, spleen and thymus). The anti-SRBC IgM concentration was measured with Enzyme Linked Immunosorbent Assay (ELISA).

There were no premature deaths and no treatment-related clinical signs. No treatment related effects on food and water consumption, hematology parameters, and gross pathology in all treated groups. When compared to the animals in control group, animals in 1000 ppm group had slight treatment-related decrease in body weight (↓2.6 – 4.1%) from study Days 13-29, and body

weight gain (↓ 8.5 – 10.8%) from study Days 7-29. The absolute and relative liver weights were increased (↑12.5% and 17.3%, respectively) and the absolute and relative thyroid weights were increased (↑20.0% and 25.6%, respectively). There were no treatment related effects on spleen and thymus weights in treated groups. Positive control group treated with cyclophosphamide had decreased body weight and body weight gain (↓3.8% and 8.6%, respectively) on study Day 29. In this group absolute and relative spleen weights were significantly decreased (↓50.1% and 48.2%, respectively); absolute and relative thymus weights were also significantly decreased (↓78.7% and 78.0%, respectively).

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The Natural Killer (NK) cells activity was not evaluated in this study. The toxicology database for Mancozeb does not reveal any evidence of immunotoxicity. The overall weight of evidence suggests that the chemical does not directly target the immune system. Under HED guidance a NK cell activity assay is not required at this time.

The NOAEL for immunotoxicity was 1000 ppm (equivalent to 81 mg/kg/day), the highest dose tested. The immunotoxicity LOAEL was not established.

This immunotoxicity study is classified **acceptable/guideline** and satisfy the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in rats.

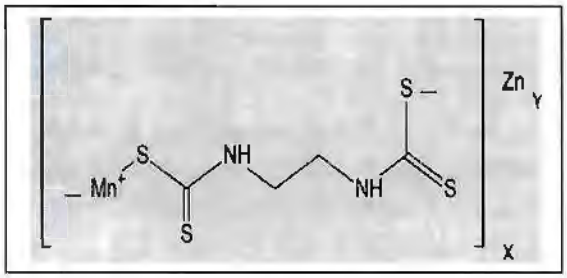
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Mancozeb

Description:	Did not mention
Lot/Batch #:	ZA0344n615. TSN 300199
Purity:	84.8 %
Compound Stability:	A previous stability study (Perala) demonstrated that Mancozeb is stable for at least 36 days in rodent feed at concentrations ranging from (0.42 – 8.48%). Stable for 23 days at 0.04% concentration and stable for two days at 0.0021% concentration.
CAS # of TGAI:	8018-01-7
Structure	X/Y ratio = 11



2. Vehicle and/or positive control:

Vehicle: Certified Rodent LabDiet #5002 (PMI Nutrition International, LLC, St. Louis, MO)
Positive Control: Cyclophosphamide monohydrate, Lot no. 079K1569; 100.5 % purity (from Sigma Aldrich, St. Louis, MO).

3 Test animals:

Species:	Rats, Male	
Strain:	Crl:CD(SD)	
Age/weight at treatment initiation :	Approximately 7 weeks old/ did not mention	
Source:	Charles River, Portage, Michigan.	
Housing:	Housed individnally in stainless steel cages	
Diet:	PMI Nutrition International, LLC, Certified Rodent LabDiet#5002 (meal), *ad libitum*	
Water:	Municipal water, *ad libitum*	
Environmental conditions:	**Temperature:**	22±3 °C
	Humidity:	Approximately 40-70%
	Air changes:	10-15 per hour
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	**One week**	

B. STUDY DESIGN:

1. **In life dates** – Treatment Start: November 15, 2011 End: December 13, 2011.

2. **Animal assignment**: A computerized randomization procedure was used.

Table 1. Study Design[a]			
Test group	Conc. in diet (ppm)	Actual time-weighted average dose (mg/kg/day)	No. of Male animals
1. Vehicle Control	0	0	10
2. Mancozeb	50	4.1	10
3. Mancozeb	200	16.1	10
4. Mancozeb	1000	81.0	10
5. Positive Control[b]	0	20	10

[a] Information was obtained from page 10 of the study report
[b] Positive Control group received Cyclophosphamide IP. 20 mg/kg bw/day from Days 24-28

3. **Dose selection:** The dose levels were selected based on the results from the 28 and 90 – day studies in rats showing decreased body weights and thyroid toxicity (increased thyroid weights, changes in thyroid hormones and histopathology) at 1000 ppm.

4. **Diet preparation and analysis:** A premix (concentrated test material and feed mixture) was mixed with ground feed to obtain the desire concentration.

Results

Homogeneity analysis: the homogeneity was determined concurrently with concentration analyses. The relative standard deviations were between 1.2% and 5.9% indicating that the diets were homogeneously mixed.

Stability analysis: It was mentioned that a previously conducted stability study (Perala, 2012), 23 days of stability was established for 400 ppm.

Concentration analysis: The concentrations of all dose levels were in the ranged from (95.7% to 100.4 %) of Target concentrations.

5. **Statistics:** Body weights, feed consumption, organ weights, hematologic data and the SRBC ELISA data was evaluated by Bartlett's test. Parametric and nonparametric analysis of variance (ANOVA) was used depending on the outcome of Bartlett's test. If significant at alpha = 0.05, the ANOVA was followed by Dunnett's test or the Wilcoxon Rank-Sum test.

C. **METHODS:**

1. **Observations:** Animals were inspected at least once a day for treatment related health effects. Detailed physical examination was done once every week.

2. **Body weight:** Body weights were recorded weekly before treatment started, on the day of treatment commenced and twice weekly throughout the study period and before necropsy.

3. **Food/water consumption and compound intake:** During the test period, food intake and water consumption were recorded weekly.

4. **Sacrifice and pathology:** On the study Day 29, animals were sacrificed by carbon dioxide inhalation followed by decapitation. Blood was collected from the orbital sinus for hematology tests and IgM antibody analysis.

a. **Gross necropsy:** A limited gross necropsy was conducted on all animals. Following gross examination, spleen, liver, and thymus, weights were recorded and placed in 10% buffered formalin solution. Also the thyroid glands and parathyroid glands were weighed post-fixation from all animals.

b. **Tissue preparation/histopathology:** did not perform.

5. **Immunotoxicity:**

a. **Enzyme Linked Immunosorbent Assay (ELISA):** On Day 24, all animals in all groups received a single intravenous dose of 0.5 mL SRBC (4 x 10^8 SRBC/ mL). On Days 24-28, the positive control group received IP cyclophosphamide 20 mg/kg/day. On Day 29, after the sacrifice, serum samples were analyzed for anti-SRBC IgM using ELISA kit (Life Diagnostics, West Chester, Pennsylvania).

b. **NK cell Assay:** Did not perform NK cell assay.

II. RESULTS:

A. OBSERVATIONs:

1. **Clinical signs of toxicity:** No clinical signs of toxicity were observed.

2. **Mortality**: There were no unscheduled mortalities during the study.

B. Body weight and weight gain: There was no treatment related effect on body weight at 200 and 50 ppm. When compared to the animals in control group, animals in 1000 ppm group had slight treatment-related decrease in body weight (↓2.6 – 4.1%) from study Days 13-29, and body weight gain (↓8.5 – 10.8%) from study Days 7-29 (Tables 2&3). Positive control group treated with cyclophosphamide had decreased body weight and body weight gain (↓3.8% and 8.6%, respectively) on study Day 29; these changes were considered related to IP injections of CP on study Days 24-28

TABLE 2. Mean body weights (g) ± SD					
Study Day	**Vehicle Control**	**50 ppm**	**200 ppm**	**1000 ppm**	**CP**
1	205.5±11.1	205.2±10.9	208.1±9.6	208.5±10.9	207.2±9.9
7	260.0±18.0	258.6±16.1	259.9±9.1	257.1±17.0	263.3±12.5
13	318.1±25.4	314.6±21.7	314.8±11.9	309.9±23.0	322.8±18.1
19	350.2±33.8	345.8±26.6	344.6±13.2	338.9±26.1	357.5±22.0
29	407.5±44.4	404.4±35.9	402.0±19.6	390.8±34.5	391.9±26.0

Data obtained from pages 46 and 47 in the study report.
There were no statistical differences from control at Alpha= 0.05
CP = Cyclophosphamide IP. 20 mg/kg bw/day from Day 24-28

Table 3. Summary of Body Weight Changes (g)					
Study Day	**Vehicle Control**	**50 ppm**	**200 ppm**	**1000 ppm**	**CP**
1-7	54.5±6.2	53.4±6.7	51.7±3.8	48.6±7.2	56.1 ±3.2
1-13	112.6±16.0	109.4±13.7	106.7±6.9	101.4±13.2	115.6±9.5
1-19	144.7±25.0	140.6±19.7	136.5±9.0	130.4±16.5	150.3±13.8
1-29	202.0±36.4	199.2±29.0	193.9±16.6	182.3±25.4	184.7±19.8

Data obtained from pages 45-46 of the study report
There were no statistical differences from control at Alpha= 0.05

C. FOOD/WATER CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption/ Food Efficiency:** No treatment related effect on food consumption.

2. **Compound consumption:** The compound consumption in each group was shown in Table 1.

D. GROSS NECROPSY: During the gross observations no treatment related visible lesions were found at any concentration.

1. Organ weight: At 1000 ppm, absolute and relative liver weights were increased (↑12.5% and 17.3%, respectively), and the absolute and relative thyroid weights were increased (↑20.0% and 25.6%, respectively). There were no treatment related effects on spleen and thymus weights in all treated groups (Table 4). In the positive control group treated with cyclophosphamide, absolute and relative spleen weights were significantly decreased (↓50.1% and 48.2%, respectively); and absolute and relative thymus weights were also significantly decreased (↓78.7% and 78.0%, respectively) (Table 5).

Table 4. Final Body Weights, Absolute and Relative Organ Weights, Mean Values

DOSE PPM		FINAL BODY WT. (G)	LIVER (G)	LIVER (G/100)	SPLEEN (G)	SPLEEN (G/100)	THYMUS (G)	THYMUS (G/100)	THYROID GLAND (G)	THYROID GLAND (G/100)
0	MEAN	407.5	16.737	4.087	0.788	0.193	0.520	0.127	0.0175	0.0043
	S.D.	44.4	3.006	0.406	0.103	0.016	0.177	0.039	0.0025	0.0007
	N=	10	10	10	10	10	10	10	10	10
50	MEAN	404.4	17.118	4.231	0.793	0.196	0.494	0.121	0.0175	0.0043
	S.D.	35.9	2.026	0.280	0.131	0.025	0.123	0.023	0.0036	0.0004
	N=	10	10	10	10	10	10	10	10	10
200	MEAN	402.0	17.319	4.298	0.792	0.197	0.404	0.100	0.0197	0.0049
	S.D.	19.6	1.907	0.303	0.062	0.023	0.077	0.019	0.0025	0.0005
	N=	10	10	10	10	10	10	10	10	10
1000	MEAN	390.8	18.821	4.797*	0.839	0.214	0.412	0.106	0.0210	0.0054*
	S.D.	34.5	2.852	0.366	0.190	0.042	0.098	0.025	0.0041	0.0009
	N=	10	10	10	10	10	10	10	9	9

Data obtained from page 55 of the study report

* Statistically significant from control mean by Dunnett's test, Alpha = 0.05.

Table 5. Final Body Weights, Absolute and Relative Organ Weights, Mean Values

DOSE PPM		FINAL BODY WT. (G)	SPLEEN (G)	SPLEEN (G/100)	THYMUS (G)	THYMUS (G/100)
0	MEAN	407.5	0.788	0.193	0.520	0.127
	S.D.	44.4	0.103	0.016	0.177	0.039
	N=	10	10	10	10	10
20 CP# MG/KG	MEAN	391.9	0.393*	0.100*	0.1115	0.0285
	S.D.	26.0	0.077	0.015	0.025	0.005
	N=	10	10	10	10	10

Data obtained from page 56 of the study report

* Statistically different from control mean by Dunnett's test, Alpha = 0.05

$ Statistically different from control mean by Wilcoxon test, Alpha = 0.05

CP = cyclophosphamide, positive control group.

2. Histopathology: Did not perform.

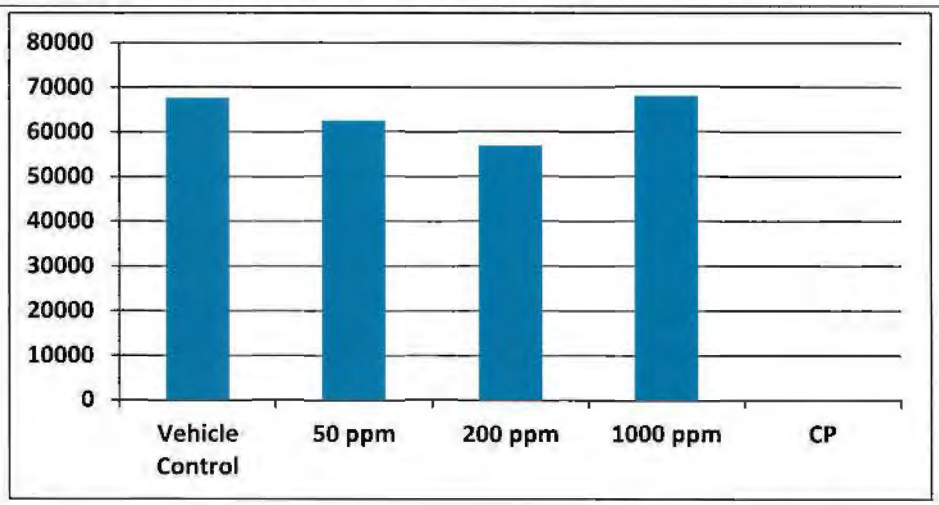
E. IMMUNOTOXICITY TESTS:

a. Enzyme linked immunosorbent Assay (ELISA): There were no statistically significant differences observed in anti-SRBC IgM levels in treated groups when compare to the vehicle control group. High inter-individual variability was noted in all the treatment groups as well as in the control group. Evaluation of the individual animal data of this study did not show any trend or distribution that would demonstrate significant suppression of anti-SRBC antibody response. Positive control group had statistically significant decrease of the antibody response (Table 6, Figure 1). This confirmed the ability of the test system to detect immuno-suppressive effects and confirmed the validity of the study design.

Table 6. ELISA Results for Anti-SRBC IgM Response	
Dose	**Anti-SRBC IgM ± SD (U/mL)**
Vehicle control - 0 ppm	67629±72906
50 ppm	62385±71402
200 ppm	56810±65854
1000 ppm	67968±79432
Positive Control (CP)	189*±77

Data obtained from pages 58-59 of study report
CP = cyclophosphamide 20 mg/kg/day I.P. from study Day 24-28
There were no statistical differences from control at alpha = 0.05
* Statistically different from control mean by Wilcoxon's Test, Alpha = 0.05

Figure 1. ELISA Results for Anti-SRBC Antibody Response



Data obtained from pages 58 - 59 of the study report
CP = cyclophosphamide 20 mg/kg/day I.P. from study Day 24-28

b. NK cell assay: Did not perform NK cell assay.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** It was reported that the Mancozeb did not reveal any signs of immunotoxicity when administered in the diet over a period of 29 days to Male Crl:CD(SD) rats. The NOAEL for immunotoxicity was 1000 ppm (81.0 mg/kg/day), the highest dose administered in this study.

B. **REVIEWER COMMENTS:** There were no premature deaths and no treatment-related clinical signs. No treatment related effects on food and water consumption, hematology

parameters, and gross pathology in all treated groups. When compared to the animals in control group, animals in 1000 ppm group had slight treatment-related decrease in body weight (↓2.6 – 4.1%) from study Days 13-29, and body weight gain (↓8.5 – 10.8%) from study Days 7-29. The absolute and relative liver weights were increased (↑12.5% and 17.3%, respectively) and the absolute and relative thyroid weights were increased (↑20.0% and 25.6%, respectively). There were no treatment related effects on spleen and thymus weights in treated groups. Positive control group treated with cyclophosphamide had decreased body weight and body weight gain (↓3.8% and 8.6%, respectively) on study Day 29; these changes were considered related to IP injections of CP on study Days 24-28. In this group absolute and relative spleen weights were significantly decreased (↓50.1% and 48.2%, respectively); absolute and relative thymus weights were also significantly decreased (↓78.7% and 78.0%, respectively).

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The NOAEL for immunotoxicity was 1000 ppm (equivalent to 81 mg/kg/day), the highest dose tested. The immunotoxicity LOAEL was not established.

C. **STUDY DEFICIENCIES:** No major deficiencies were noted.